RESIDUE STUDIES WITH A NONIONIC SURFACTANT USED FOR BLOAT CONTROL IN DAIRY CATTLE AND ITS EFFECT ON RUMEN FERMENTATION, FEED INTAKE, AND MILK PRODUCTION

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INTRODUCTION

During the past century, and particularly in the past twenty-five years, the incidence of bloat has been enhanced by an increase in the use of pure legume and mixed legume pasture. The losses resulting from bloat are becoming of great economic importance. It is estimated that this disorder results in annual losses of over $40,000,000 in the United States. This figure considers the estimated loss of production resulting from restricted use of legume pastures in areas where bloat prevails as well as the number of cattle and sheep dying from bloat.

Although it is apparent that all the facts concerning the bloat complex have not yet been revealed, the information available strongly suggests that bloat is a problem of the development of foam or froth in the rumen. According to Bartley and Yadava (1961), the following conditions seem to be necessary for frothy bloat to occur: consumption of plants containing active foaming agents, conditions in the rumen suitable for stable foam formation, gas production, and finally the lack of sufficient quantities of the antifoaming factor mucin due to reduced salivation by animals grazing succulent forage.

Since bloat is essentially a problem of the development of foam in the rumen, it would appear that bloat control should be afforded by the use of antifoaming agents. Although several antifoaming agents such as silicones, detergents, vegetable oils, tallows, and liquid paraffins have been used with some degree of success, the natural forms of even the more effective of these materials have not proven entirely satisfactory.

Choice of an antifoaming agent for field use must take into consideration not only the effectiveness of the agent in preventing bloat, but also the possible effects on the health of animals and the quality and

quantity of milk and butterfat produced, the ease of administration, and the cost.

The results of studies conducted at the Kansas Station using anti-foaming agents specially tailored for conditions of the rumen demonstrated that a surfactant effectively prevented bloat during the first 12 hr after administration. This agent has been effective at low, economical levels and, by incorporating it into a grain mixture, can be easily administered. The experiments described in this thesis were designed to determine the effect of the surfactant on animal health and reproduction, feed consumption, rumen fermentation, and quality and quantity of milk and butterfat produced. The following aspects of rumen fermentation were studied: cellulose digestion, pH, ammonia and volatile fatty acid concentration.

REVIEW OF LITERATURE

Cause and Effect of Foaming in Legume Bloat

Mechanism of Foam Formation. Bloat is frequently classified as either chronic or acute. Cole et al. (1945) define chronic bloat as bloat extending over a considerable period due to an abnormal physiological state of the animal, and acute bloat as bloat that occurs because of an abnormal feeding regime that in some way interferes with the animal's ability to expel gas. Acute bloat is classified as "frothy" or "free gas" bloat according to whether there is foam or free gas present in the rumen. Johns (1954), however, believes that the supposed differences between "frothy" and "free gas" bloat may be due only to the degree of foaming, and that the amount of free gas present is a function of the stability of the foam.

Since the stability of the foam is dependent on the consistency of rumen fluid, many workers believe that surface tension is involved. Weiss (1953) found that when the rumen contents were watery, the gas-bubbles rose freely to the surface to form a layer of unstable free foam on the top, but no bloat was observed. As the consistency of the rumen fluid increased, there was a greater tendency for the gas bubbles to become entrapped in the thick viscid material, and for the ingesta to rise up into a frothy mass causing bloat. Similar observations were reported by Hungate et al. (1955) and Johns (1956). Johns (1954) observed that substances which reduced the surface tension of water tended to promote foam formation so that when fermentation took place within the liquid, the gas produced was retained within the froth formed. The quantity of gas retained depended on the stability of the foam. The degree of bloat, therefore, depended on the relation between the rate of gas produced per unit of time and the rate of release from the rumen contents. If sufficient foaming agent was present, release of gas was much slower than gas production and severe bloat resulted.

Although many investigators agree that surface active agents can be used to alter surface tension, they differ in their opinions as to the action of these agents in the treatment of bloat. One school of thought is that intrarumen foam is formed because rumen liquids have a very low surface tension; therefore, to break a foam an agent should be added that will raise the surface tension and thereby reduce the ability of the liquid to foam. Others believe that even though liquids of low surface tension are more prone to foam, foams that are formed when there is a low surface tension are unstable and will break readily allowing the gas to escape.

These conflicting views on the mechanism of foaming have led to some confusion as to the manner in which antifoaming agents affect foaming.

For example, the beneficial effect of turpentine, which is a common agent for the treatment of bloat, has been attributed by Clark (1948) to its ability to break foam by increasing surface tension, but Blake et al. (1955, 1957) have since shown that turpentine decreased the surface tension of ruminal ingesta.

Johns (1956) stated that there are two broad groups of foaming materials: (1) solutions of soaps and detergents in which the surface tension is considerably lower than that of water and which exhibit low viscosity and produce foams which, generally, are not particularly stable; (2) proteins and saponins in the presence of suitable metal salts which exhibit strong superficial viscosity and yield foams of great stability and at the same time exhibit fairly high surface tension values which show little correlation to foam stability.

Johns (1956) explained that the surface viscous and non-viscous types are mutually incompatible so if soap and saponin are mixed, the soap, having the lower surface tension, causes displacement of the saponin from the surface layer. This reduces the viscosity of the system and gives a more unstable foam. In general, antifoaming agents act by displacing the foaming substances from the surface of the foaming liquid by providing a nonfoaming surface layer. The effectiveness of some surface active agents and detergents in the treatment of frothy bloat was demonstrated by Nichols (1954) and has since been investigated by many other researchers.

Nichols (1954) observed also that the feeding of fresh legumes reduced the effective buoyancy of rumen fluid with the amount of reduction being directly related to the quantity of gas bubbles formed on the small particles of feed in the ingesta. Since each feed particle and each attached bubble displaced an equal volume of liquid upward, the fluid level of rumen ingesta

in bloated animals was higher than in animals fed grass or hay. Because of the decreased buoyancy of the rumen fluid, recently eaten fresh legumes quickly gravitated into the ingesta and did not remain near the top as was the case with hay just after it had been fed. Nichols observed that frothing appeared to start in the upper regions of the paunch and extend into the lower regions where it increased in intensity.

Feeding fresh alfalfa, according to Nichols et al. (1956) caused an increase in surface tension and a decrease in viscosity of rumen fluid, whereas feeding alfalfa hay caused a reduction in surface tension and little change in viscosity. Animals with the driest paunch contents and consuming the least water had the highest surface tension and viscosity values.

The Effect of Plant Factors on Foaming. Although several plant components are believed to be involved in froth formation, the role of plant saponins in foam formation has been more intensely investigated than any of the other plant factors. There seems to be no doubt that saponins play a role in the foaming of ruminal ingesta, and, thus, in the production of bloat.

Lindahl and Davis (1957), using sheep, appear to be the first to have produced bloat with a saponin isolated from a pasture legume. In vivo and in vitro experiments show that alfalfa saponin contributes to the formation and stabilization of froth of ruminal ingesta. The results of these studies indicate that the isolated alfalfa saponins have pronounced physiological effects in addition to their effects on surface tension. It was evident, however, that alfalfa saponin was not the only factor involved in stable foam formation. Severe ruminal distension was produced only when the animals were given alfalfa saponin at near toxic levels.

There have been some attempts to isolate the foaming factor(s) from fresh legumes. Ferguson and Terry (1955) fractionated bloat-provoking lucerne juice to show that it was still capable of producing bloat after precipitation of chloroplastic material and passage of the clear juice through an anion or cation exchange resin. This suggests that the factor(s) causing bloat are nonionic and not absorbed on resins. Similar properties are exhibited by saponins.

It is the opinion of Weiss (1953) that the presence of saponins in legumes is not the cause of bloat per se, but it merely contributes towards the colloidal state of the ingesta, thus making conditions more favorable for foaming. Gutierrez et al. (1958), on the other hand, presented evidence that alfalfa saponins were utilized by certain rumen bacteria with resultant production of acids, gas, and large amounts of slime, all of which may be involved in foaming.

Plant proteins also are believed to be of some importance in the foaming of rumen ingesta. This view is supported by the work of Boda et al. (1957) who added eggwhite to a ration containing dehydrated alfalfa. A definite increase in intrarumen pressure resulted due to the formation of a stable foam.

Head (1959) found that foam taken directly from the rumen contents of a cow grazing clover or lucerne contained sugars of pectin and hemicellulose (non-protein, non-nitrogenous substances) as the main foaming constituents. Bartley and Bassette (1961), however, using essentially Head's method of isolating foaming constituents, found that the frothing agents present in rumen foam obtained from animals bloating on alfalfa pasture were primarily proteinaceous in nature. The difference in these two results can possibly be attributed to differences in the two foams analyzed.

Work of Warner et al. (1959) showed that urea and sugar solutions sprinkled on chopped green forage just prior to feeding increased the incidence of bloat slightly. However, bloat frequency was not affected by additions of eggwhite alone or in combination with glucose. Other studies by Warner et al. (1962) indicated that the foliar application of urea (24-48 hr prior to grazing) at the equivalent rate of 40 lb per acre significantly increased the severity of bloat, but lower levels had no appreciable effect. The foliar application of glucose increased the degree of bloat in some comparisons, but not in others. Total nitrogen and NaOH (0.02N) soluble nitrogen of alfalfa tops were increased by foliar application of urea. Although blood ammonia nitrogen and non-protein nitrogen were higher when the forage was treated with urea, there was no consistent agreement between blood nitrogen values and severity of bloat.

Conrad et al. (1958) have shown that the plant substances responsible for the initial rapid gas production in ingested alfalfa were closely associated with the fiber portion of the plant, but that these substances were removed after 4-10 hr of fermentation by microorganisms or by extracting for 12 hr with hot water, indicating that materials other than cellulose were the principal precursors of the gas produced. Results indicated that the combined effects of the physical structure of green alfalfa, its pectic substances and galacturonic acid produced by hydrolysis of pectic substances, were capable of causing formation of stable foam found in legume bloat.

Pressey et al. (1963b), however, stated that although pectins were capable of serving as foam stabilizing agents and, in this way, were possibly related to bloat, the level of pectic substances present in legumes does not appear to be related to degree of bloat.

Other investigators, Smith and Woods (1962a, 1962b) and Warner (1962), have shown that plant minerals such as calcium and magnesium, which are present in large proportions in legumes, may also be important in the etiology of bloat.

The results of most experiments indicate that no single factor is solely responsible for the formation of rumen froth, but that the cause of legume bloat may be due to the interaction of several units of the legume plant cell.

The Effect of Saliva on Foaming. Although most investigators now support the hypothesis that saliva plays a beneficial role in bloat, several workers have attributed the role of saliva in bloat to one of promoting foaming.

Johns (1954) compared the surface tension of water (71.3 dynes per cm), with that of saliva (47.1 dynes per cm) and concluded that the low surface tension of saliva was one factor that would augment foaming. Blake et al. (1957a), however, found in testing the effect of various compounds on the surface tension of rumen fluid, that water and saliva were the only materials that raised surface tension. Johns (1954, 1956) along with Reid (1959) presented evidence that the mucoproteins present in saliva may assist in forming a stable, viscous type foam.

Results of work reported by Van Horn and Bartley (1961) and Bartley and Yadava (1961), however, suggested that saliva serves as a foam inhibiting agent when associated with the foam forming constituents of bloat provoking legumes. Saliva added to incubated frothing rumen contents permitted greater quantities of gas to escape than when no extra saliva was administered. Mucin appeared to be the component of saliva responsible for its foam breaking

action. Fina et al. (1961) isolated five strains of rumen bacteria capable of breaking down salivary mucin and introduced cultures of these organisms into the rumens of fistulated cows grazing a mature nonbloat-provoking legume pasture, or consuming feedlot rations which were subbloat-provoking. Since bloat resulted in the majority of instances, it was postulated that mucin in normal salivary secretion, if sufficient in quantity, prevented bloat, but reduced salivation or destruction of mucin by excessive concentrations of mucinolytic flora may result in bloat.

Many workers have investigated the factors influencing reflex salivary secretions. Ash and Kay (1959) and Kay (1958) found that stroking the area around the cardiac orifice was the most effective stimulus to evoke reflex parotid secretion. Weiss (1953) believes that the formation of rumen foam depends on the consistency of ruminal ingesta, which in turn is influenced by reflex salivation. Bloat caused by foaming of thick, viscid, ruminal ingesta occurred immediately after succulent, leafy alfalfa was fed. When mature, stalky alfalfa was fed, the ruminal ingesta reverted to a watery consistency and bloat ceased. He attributed this effect to reflex salivation caused by coarse, stemmy material scratching the cardia and the walls of the forestomach around the cardia. Bailey and Balch (1961) and Balch (1958) also have shown that fibrous feeds such as hay stimulated much more secretion of saliva per unit weight of feed consumed than did less fibrous feeds such as fresh grass.

In order to test the effect on bloat of a drug that inhibits salivation, Yadava (1960) injected atropine sulfate subcutaneously (50 mg per cow per day) and the reduction in saliva production resulted in increased rumen foam in

members of identical twins grazing mature alfalfa. Mendel and Boda (1960) observed that nonbloaters secreted greater quantities of saliva than did bloaters.

Mead et al. (1944) and Cole et al. (1943) showed that feeding coarse fibrous materials such as sudan hay or barley straw prior to grazing bloat-provoking legumes prevented bloat. These workers believe that scabrous materials may prevent bloat in the following ways: (1) they physically prevent the formation of froth; (2) they contain a chemical antifrothing agent; (3) their scabrous nature causes stimulation of the eructation reflex. These workers did not consider reflex salivation as one of the beneficial effects of roughage feeding. Weiss (1953), however, maintained that the physical condition of the feed consumed has a direct bearing on the occurrence of bloat through its action on reflex salivation rather than its effect on the eructation reflex.

Kay and Phillipson (1959) and Phillipson and Reid (1958) found there were individual differences in saliva flow when pressure was produced in the rumen. They found that pressure in the rumen usually caused a marked increase in the rate of secretion of parotid and residual saliva. The pressure required to cause this stimulation varied from 8-20 mm Hg. Once stimulation occurred, further increase in rumen pressure caused inhibition of salivary secretion. Some animals, however, did not increase salivary flow in response to rumen pressure stimulation. Since these workers were advocates of the theory that saliva contributes to foaming, they suggested that the additional salivary flow in response to pressure in the rumen may be important in causing a moderately bloated animal to become worse.

The Effect of Bacterial Slimes on Foaming. Hungate et al. (1955) suggested the possibility that some cellulolytic rod shaped strains of

microorganisms present in rumen contents were capable of producing a mucilaginous material or slime which could slow down bubble movement and thereby contribute to stable foam formation. Gutierrez et al. (1958) presented evidence that alfalfa saponins and soluble sugars in plant materials were utilized by rumen bacteria with resultant production of acids, gas, and large amounts of bacterial polysaccharide slime. The latter formed a stable foam in which the rumen fermentation gases were entrapped in the ingesta as numerous small gas bubbles. Gutierrez and David (1962) isolated saponin-utilizing bacteria from the rumens of cattle bloating on Ladino clover pasture. They observed that the slimy residue in the saponin bacterial cultures did not resemble the slimy fraction which had previously been isolated from the rumens of steers bloating on a high grain ration.

Jacobson et al. (1957a) observed that rumen samples from animals grazing blue grass-white clover pasture produced stable foam in vitro. Also it was observed that 0-16% of the microorganisms in these rumen samples were encapsulated. The degree of encapsulation increased with the length of time on bloat producing diets (Jacobson et al., 1957b). There was a highly significant correlation (0.94) between the average percentage encapsulation and the average bloat index.

The Effect of Rumen Froth on Eructation Reflex. Sheep which had been insufflated with air by Quin (1943) were capable of belching up to six liters of gas per minute. Since gas production in the rumen is much lower than gas loss by belching, it was concluded that gas production in itself was not the cause of bloating. Weiss (1953) obtained similar results when he introduced air artificially above the ruminal mass, but when air was bubbled through the ruminal ingesta of sheep fed freshly cut lucerne, there was marked

interference with eructation. This was believed to be due to the ingesta rising up in a frothy mass and mechanically interfering with eructation.

Although Olsen (1944) postulated that the deterrent to belching in bloated animals was either the blockage of the cardia by accumulated ingesta or the partial paralysis of the rumen by toxic gases, most investigators agree that bloat on fresh legumes is the result of stable rumen foam interfering with eructation.

Johns (1954) observed that belching was not inhibited while animals were becoming bloated. The frequency of belching was often greater in a bloating animal than in the same animal when it was not bloating. However, in the former instance the animal was still not able to get rid of enough gas to prevent bloat. On occasions, belching ceased in severely bloated animals, but cessation appeared to be the effect of bloating rather than the cause. Colvin et al. (1958) made similar observations in their experiments and concluded that froth formed during rapid fermentation of succulent legumes was sufficiently intense to occlude the cardia and to render the eructation reflex ineffective even though the frequency of secondary or eructation contractions should have been adequate to reduce the intraruminal pressure. When no froth was present, as was the case when alfalfa hay was fed, an eructation occurred on every secondary contraction. Rumen motility studies by Colvin et al. (1958, 1959) gave similar results and it was concluded that animals suffering from legume bloat unsuccessfully attempted to increase the number of eructations by increasing eructation contractions.

Eructation is an independent reflex mechanism with pressure receptors in the rumen wall (especially the dorsal blind sac) which are stimulated by gas pressure. Johns (1958) introduced various foams into the paunch and gas

was passed into the paunch. Evidence was obtained that the reflex receptor controlling the opening of the cardial sphincter could distinguish between free gas and foam because foam caused inhibition of belching.

The Use of Antifoaming Agents in Treatment and Prevention of Bloat

Although many methods have been used for the treatment of bloat, none has been more successful for the treatment and prevention of frothy bloat than the use of antifoaming or surface active agents. Dougherty and Courtney (1954) believe that surface active agents should relieve most cases of bloat and would justify extensive use of them should the following conditions exist in bloated animals: (1) if all (or most) bloat were frothy in nature; (2) if there were no inhibition, other than mechanical, of the eructation mechanism; (3) if all surface active agents were active in all or most cases of bloat; and (4) if wide dispersal of the agent in a severely distended rumen were possible. In choosing an antifoaming agent for field use, one must consider not only effectiveness of the agent in preventing bloat, but also the possible effects on the health of animals and the quality and quantity of milk and butterfat produced, the ease of administration, and the cost.

Although turpentine was among the first antifoaming agents to be used to control bloat, it was not until Clark (1948) showed that its effectiveness in the treatment of bloat was due to its effect on surface tension, not on gas formation, that its mode of action was recognized. Johns (1954) and Blake et al. (1955, 1957a) showed that turpentine effectively relieved bloat by reducing the surface tension of rumen fluid. Turpentine, however,

has not proven entirely satisfactory for field use. Johns (1956) and Reid (1958) reported that turpentine caused intense irritation of the mouth and other organs, adversely affected the animal's appetite, accumulated in amounts which were toxic to animals, and caused a marked taint in milk.

Upon evaluating the antifoaming properties of turpentine products with those of silicone products in vitro, Dougherty and Courtney (1954) found that the efficacy of the silicones was higher than the turpentines, based on foam dispersal, but the reverse was true when the products were judged on ability to prevent reconstitution of foam when the rumen liquor was re-shaken. Although Quin et al. (1949) believed that methyl silicone relieved bloat by increasing surface tension of ruminal ingesta, the experimental data accumulated by Blake et al. (1957a) demonstrated that all antifoaming agents employed (including methyl silicone) decreased the surface tension of rumen fluid. Nichols et al. (1957) examined the effects of twenty surface active agents on properties of rumen fluid and observed that silicone was not reliable in surface tension reduction, but gave good control of mechanical foaming.

The recovery rate of 155 cases of bloat in cattle treated by Quin et al. (1949) with a highly polymerized methyl silicone was approximately 80% when the agent used was in tablet form and 95% when an injectable, ready-to-use suspension was employed. Smith et al. (1953) and Johns (1954) also found methyl silicone to be effective in reducing froth while Hungate et al. (1955) reported that 20 g of methyl silicone administered in capsules to animals gave some protection against bloat for 5-6 hr. Methyl silicone, however, was not completely effective in preventing bloat in the experiments of Jacobson et al. (1957a).

In evaluating the usefulness of silicones as antifoaming agents in controlling bloat, both Johns (1956) and Reid (1958) note that while silicones are efficient foam breakers, they are not always reliable in treating bloat. Johns (1956) observed that silicones have low toxicity in animals, but are too expensive for practical use in controlling bloat.

Since mucin is believed to be the component of saliva responsible for its foam breaking action, Bartley (1957) evaluated the effectiveness of linseed mucin in reducing the incidence and severity of bloat in cattle. Mucilaginous extracts from linseed meal added to frothing rumen contents from bloated fistulated cattle resulted in a greater release of gas when incubated at 39 C for 2 hr than rumen contents incubated alone. Feeding linseed meal to cows before pasturing appeared to reduce the incidence of bloat. These results were confirmed in experiments conducted by Van Horn and Bartley (1959). Since the extraction of mucilage from linseed meal is a very difficult and costly process, linseed meal mucilage was not recommended as a practical means of controlling bloat.

Bartley and Yadava (1961) tested in vitro the antifoaming activity of bovine saliva, plant mucilages, and animal mucins on alfalfa saponin foams. Bovine saliva and two animal mucins effectively inhibited foam formation. In vivo evaluation of the two animal mucin products indicated that levels of 50 or 75 g effectively prevented bloat for 2 to 4 hr. Since neither of these products prevented bloat for more than 4 hr, they are not presently endorsed for field use. Johnson et al. (1958) found that gastric mucin (50 or 100 g per feeding) was unpalatable and did not reduce bloat in steers. Both levels seemed to increase foaming.

Smith and Woods (1962a, 1962b) have shown that certain chelating agents reduced the severity of bloat by decreasing the ruminal concentration

of ionic calcium, magnesium, and ammonia. Early results of Smith and Woods (1962a) indicated that diamine tetraacetic acid (DTPA) was more effective in reducing bloat than ethylene diamine tetraacetic acid (EDTA) when these agents were introduced directly into the rumens of bloated animals. Later investigations by these workers (1962b) revealed that drenching lambs with 1.25 g of chelating agents, DTPA or ethylene diamine di-(o-ohydroxphenyl) acetate (EDDHA) prior to morning grazing reduced bloat 17 and 20% respectively. Feeding lambs 1.25 g of EDDHA in a soybean meal carrier prior to grazing reduced bloat 46% as compared to lambs fed soybean meal alone. Bloat was reduced to a similar degree in a trial comparing drenching and feeding EDDHA.

Many types of oil compounds and their derivatives have been used to reduce and control frothy bloat. Oils, like other antifoaming agents, reduce bloat by lowering the surface tension of rumen ingesta. Johnson et al. (1956) reported that the surface tension of rumen fluid from steers grazing alfalfa pasture and receiving a water-dispersible oil at a level of 1% in the drinking water was 57.2 dynes per cm while that from control animals was 63.7 dynes per cm. Despite the apparent ability of oils to lower surface tension, Pressey et al. (1963a) observed that small amounts of crude soybean oil increased foam stability slightly. Higher levels, however, inhibited foam stability.

Southcott and Hewetson (1958) showed that peanut oil on drinking water (12 oz of oil per head per day), or on the drinking water and on a hay supplement (14 oz of oil per head per day), reduced the incidence and severity of non-fatal bloat in cattle grazing clover-rich pasture, but failed to prevent sporadic deaths.

Johnson et al. (1958a) found that soybean oil, lard oil, and lecithin mixed with soybean oil greatly reduced bloat in steers on alfalfa pasture for several hours when fed at the rate of 0.25 lb or more per animal per feeding in grain or at the rate of 2% in drinking water. There appeared to be some carryover effect of treatment from morning to evening. Crude soybean oil sprinkled on chopped alfalfa soilage at a level of approximately 0.25 lb per 1000 lb body weight per animal per day was also effective in controlling bloat. The oral administration of 150-250 ml of lard oil successfully relieved severe cases of bloat causing the release of large quantities of gas from the rumen via stomach tube and/or eructation soon after administration.

Rumen fluid samples taken via stomach tube 2-3 hr after lard oil or soybean oil were fed in the grain or on the forage or when lard oil was fed in the drinking water to animals that were on alfalfa pasture and soilage showed a marked reduction in surface tension according to Brown et al. (1957). Although these investigators were unable to show that these treatments had any apparent effect on foam volume, foam stability, or ingesta volume increase, they were also unable to find any apparent relationship between these rumen fluid characteristics and the severity of bloat. There was, however, a positive correlation between bloat severity and ammonia content of ingesta from untreated animals, but treatments which caused considerable reduction in bloat did not reduce rumen ammonia values.

Johnson et al. (1958b) observed that bloat in cattle grazing alfalfa pasture and receiving 0.25 lb per feeding of soybean oil in 1.5 lb of a simple grain mixture was controlled for several hours. Ground soybeans (natural oil content approximately 0.25 lb per feeding) failed to reduce

bloat. Both lard oil and soybean oil (100-200 ml) administered by stomach tube gave prompt relief in severe cases of bloat if administered before the animals were moribund.

Brown et al. (1958), testing a water-dispersible oil (a lard derivative) observed that a relatively large quantity of this oil (1-2%) was needed to control bloat. Oral administration of 1 lb of oil per head per day to animals grazing alfalfa pasture completely prevented bloat for several hours, whereas seven of ten control animals bloated (two died). Injecting 100 ml of lard oil intraruminally apparently brought about a release of gas from the ingesta to the free space above it, as treated animals either began to eructate gas voluntarily in 15-40 min, or passage of a stomach tube brought release of large quantities of free gas. Spraying 0.25 lb of either water-dispersible lard oil or soybean oil (per animal per day) on fresh alfalfa soilage also resulted in reduced bloat.

Both Boda (1958) and Colvin et al. (1959) reported that vegetable oil (Wesson) administered during the development of bloat increased the rate of eructation and the volume of gas expelled and reduced intraruminal pressure within 15 min. Colvin et al. (1959) also observed that fat intakes of 0.06 lb per head per feeding before alfalfa feeding prevented bloat for the period immediately following their administration, but even at intakes of 0.5 lb per head per day, these effects did not carry over into subsequent feeding periods.

Johnson et al. (1960) and Johnson and Allen (1960) tested an inexpensive, water-dispersible antifoaming agent consisting of two parts of crude soybean oil and one part of an emulsifier composed of casein, sodium carbonate, ethanol, and water. Johnson and Allen (1960) found that in 90 of 95 cases

of bloat in which relief could not be effected by stomach tube alone, administration of 100-400 ml of this product by stomach tube resulted in prompt release of large quantites of gas (via stomach tube and/or eructation), usually in less than a minute. No adverse side-effects were observed and the animals resumed grazing within 15-40 min. The emulsifiable oil was found to be effective more promptly than non-emulsifiable oil.

Van Horn et al. (1963) utilized 221 cases of pasture bloat in comparing the effectiveness of the following treatments: crude soybean oil, emulsified soybean oil, emulsified lard oil, lard oil emulsifier, and soybean oil emulsifier. Although bloat normally was reduced following administration of each of the oils, emulsified soybean oil administered by stomach tube into the dorsal rumen was the most consistent in prompting rapid recovery. In vitro results were similar to those obtained in vivo.

Johnson et al. (1960), working with an emulsified soybean oil for bloat therapy, found that this product met the criteria of being a palatable, inexpensive, nonirritating compound with rapid, efficient bloat-preventing action. Brown et al. (1958) reported that there was no evidence that adding soybean oil or lard oil to feed lowered feed consumption, and that the addition of water-dispersible lard oil to drinking water consistently increased water consumption.

The factor limiting the effectiveness of fats and oils in preventing bloat is the rapidity with which they are lost from the rumen. Reid (1958, 1959) stated that the water immiscibility and low specific gravity of fats and oils caused them to float and concentrate at the surface of the rumen contents and probably facilitated their early transfer to the omasum.

From the standpoint of application, this does not represent a problem where the fat or oil can be added directly to the forage as in soilage

operations or where the animals can be restricted for a 2- to 3-hr period to a legume pasture after feeding concentrates containing oil or tallow. However, when animals must be pastured for longer periods, procedures must be devised to prolong their effectiveness. Reid (1958) proposed three possible solutions to this problem: (1) using dose rates high enough to counter losses between dosings; (2) absorbing the antifoaming agent on some material which will disintegrate, or will be digested only slowly in the rumen, or (3) administering small amounts frequently or arranging for a continuous intake of the antifoaming agent. Neither of the first two possibilities has been found to be practicable. The third possibility has led to the treatment of drinking water and spraying of pastures.

The addition of antifoaming agents to drinking water is unreliable according to Reid (1959) and Cole and Boda (1960) because water intake, and thus oil intake, varies greatly from animal to animal and from day to day. Reid (1958, 1959) and Johns (1959) stated that spraying of bloat-provoking pastures with emulsified peanut oil or tallow, in conjunction with strip grazing to insure ingestion of sprayed forage, provided effective and practical control of bloat. At levels normally used, (2-4 oz per cow per day) there was little or no damage to forage or to subsequent pasture growth.

Allen et al. (1959) investigated the dispersibility of I^{131} labeled soybean oil in the rumen. Poor distribution of radioactivity in rumen ingesta resulted when labeled oil was administered via fistula, but oral ingestion gave good distribution. The I^{131} activity in rumen neutral fats declined sharply with less than half the original activity remaining 4 hr after administration.

Many of the oils and fats have undesirable side-effects when fed to animals in quantities greater than 50-100 ml per day according to Cole and Boda (1960). Reid (1958) reported that mineral oils reduced the absorption of essential fat-soluble nutrients from the diet, particularly carotene and vitamins A, D, E, and K. Although dosings at irregular intervals and at rates commonly used for reducing bloat appeared to have no adverse effects, their continued use over a prolonged period seemed to be inadvisable. Dosing cows with 75 ml paraffin twice daily for 4 weeks caused a fall in blood carotene and butter tocopherol and carotene levels. Very light paraffins, such as kerosene, markedly affected feed intake, probably because of irritation of the gut. Whale oil, another cheap and effective antifoaming agent, caused a marked taint in milk and butter and adversely affected milk production and butterfat quality. Vegetable oils also caused changes in butterfat quality, though to a much smaller extent. These results were confirmed by the work of Johns (1956).

Many researchers have recognised that detergents are among the most promising of the synthetic products available for controlling bloat. Blake et al. (1956, 1957b) administered an alkyl aryl sodium sulfonate-type detergent (Ultrawet "K") via gelatin capsules at rates of 3 and 20 g daily per 1000 lb body weight to two groups of cattle grazing alfalfa. Only at the higher rate did a significant reduction in incidence and severity of bloat occur. During bloat, the ingesta volume increase, foam durability, and degree of foaminess of rumen ingesta markedly increased, surface tension and viscosity were moderately increased, while the specific gravity and pH were lowered moderately. All these properties were altered in the opposite direction (toward prebloat levels) during detergent treatment.

Nichols et al. (1957) observed that 5 g doses of alkyl aryl sulfonate and one other detergent reduced surface tension, but produced no change in viscosity. Reid (1959) and Pressey et al. (1963a) stated that low levels of Pluronic L62 were very effective in reducing foam stability. Ferguson and Terry (1955), however, were unable to show any influence by two household detergents tested on bloat produced by lucerne juice.

Several other products have also been evaluated as antifoaming agents. Blake et al. (1957a) and Pressey et al. (1963a) have shown that certain long-chain fatty acids decrease surface tension and inhibit foam stability of rumen fluid. Trisodium phosphate and cholesterol were ineffective as prophylactics for pasture and alfalfa juice bloat, according to Blake et al. (1956, 1957b). Nichols et al. (1957) found that a granular plant lecithin, 92% active in a concentration less than 20 mg per 155 ml of rumen fluid gave excellent reduction in surface tension, good control of mechanical foaming, and little evidence of any consistent alterations of gas production, sediment reaction, or cellulose digestion. Johnson et al. (1958a) observed that while 25 ml n-decyl alcohol was successful in relieving very severe cases of bloat, its effect was of too short duration for satisfactory prophylaxis of bloat. A rinoleic acid derivative was successfully employed by Johns (1954) both as a preventive and as a drench for relieving bloated animals.

In addition to the antifoaming agents mentioned here, there are also several commercial products available today for the treatment and prevention of bloat.

EXPERIMENT I

Experimental Procedure

The nonionic surfactant which has proven effective in preventing alfalfa bloat and which was tested here is a polyoxypropylene polyoxyethylene block polymer[1] (hereafter referred to as POE). The surfactant POE is a new product and all its properties have not been determined. The material is a liquid and has a high molecular weight.

To be a useful bloat preventive agent for lactating dairy cattle, an antifoaming agent must not only control bloat effectively, but must have no adverse effects on the productivity of the cow. This experiment is designed to determine the effects, if any, of POE on milk production, milk fat production, feed intake, reproduction, and animal health.

Feeding and Management. Twelve lactating cows (ten Holsteins and two Ayrshires) in the Kansas State University dairy herd were paired as similarly as possible with regard to breed, age, body weight, milk production, and fat test. The cows in each pair were placed at random in one or the other of two groups. Cows in one group were used as controls and those in the other group received POE with their grain ration. The duration of the experiment was 12 weeks.

All cows were fed 1.5 lb chopped alfalfa hay per 100 lb body weight per head per day. To obtain maximum production, the quantity grain received by each cow was established by increasing the quantity of grain fed to each cow to the maximum amount that was readily consumed. The grain ration

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[1]Smith Kline and French Laboratories, Philadelphia, Pennsylvania. Number 18667.

consisted of: ground corn, 500 lb; ground sorghum, 500 lb; soybean oil meal, 300 lb; salt, 20 lb; and dicalcium phosphate, 15 lb.

The following supplements containing the experimental treatment were placed on top of the grain fed to the cows: Supplement I, ground sorghum 74 lb, animal fat 4 lb, and POE 1.76 lb; Supplement II, ground sorghum 74 lb, animal fat 4 lb, and POE 3.52 lb. Cows in the experimental group were fed sufficient Supplement I to supply 5 g POE per cow per feeding during the first 11 days of the experiment, sufficient Supplement I to supply 10 g POE per cow per feeding during the next 38 days, and sufficient Supplement II to supply 20 g POE per cow per feeding during the final 35 days of the experiment. Cows in the control group received a supplement containing similar quantities of ground sorghum and animal fat. The supplements were added to the grain mixture fed to the cows twice daily before milking.

All cows were confined to individual stalls in the University barn from 4 PM until 9 AM each day, and were allowed to exercise in a lot near the barn during the remainder of the day. The animals were milked twice daily at 4 AM and 4 PM. Chopped hay was fed in the barn following each milking.

Records Kept and Samples Analyzed. Individual milk weights were recorded at each milking. The amount of hay and grain refused by each cow was weighed and recorded at 9 AM each day. All cows were weighed on two consecutive days every 2 weeks. Fat tests were determined on individual 48-hr composite samples (according to production) collected for the first time the day before the start of the preliminary period and thereafter at monthly intervals. Records on health and reproduction were kept on all animals.

Results

The control cows produced slightly more milk during the preliminary period and the first 2 weeks of the experiment than those receiving POE (Figure 1), but cows in the latter group produced the most milk during the last 10 weeks of the experiment. The cumulative milk production of the group receiving POE was greater than that of the control group (Figure 2). The difference in the average total production per cow between groups was approximately 200 lb of milk for the 12-week experiment. This difference was not statistically significant.

During the preliminary period, the milk of the cows receiving POE contained slightly more fat than that of the control cows (Figure 3). Also throughout the experimental period the milk of the cows receiving POE contained more fat than that of the controls. The difference in fat content was small and was not statistically significant.

Both groups of cows gained in weight during the experiment (Figure 4). The shape of the weight curves is similar for both groups throughout the experiment. The cows in the control group were slightly heavier than those receiving POE. However, this difference was not significantly different.

Feed was consumed in nearly identical amounts by both groups of cows (Table 1). Cows in the control group consumed slightly more hay and grain than those in the treatment group. More hay was refused by the cows in the treatment group, but the control cows refused the most grain. Only the difference in grain refusal approached statistical significance. Several cows went "off feed" temporarily during the first week of the trial, but the abrupt change in the roughage ration is believed to have been the cause of this since cows from both groups were similarly affected.

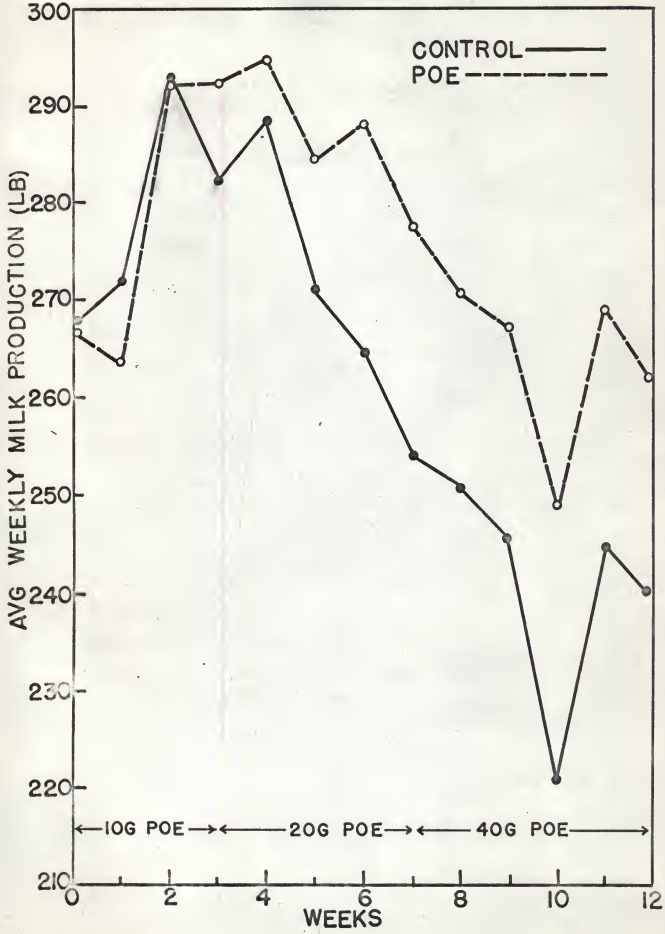

Figure 1. Effect of POE on average weekly milk production.

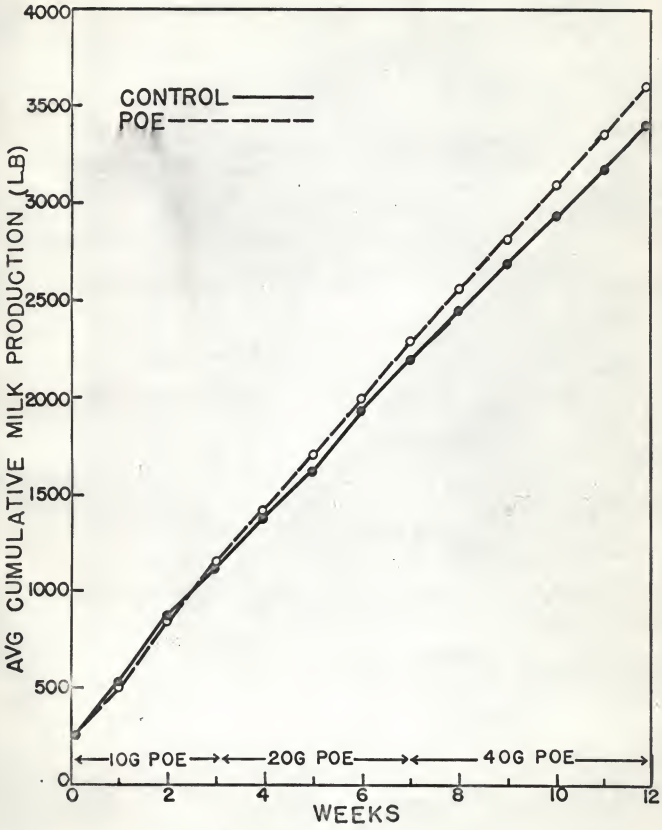

Figure 2. Effect of POE on average cumulative milk production per cow.

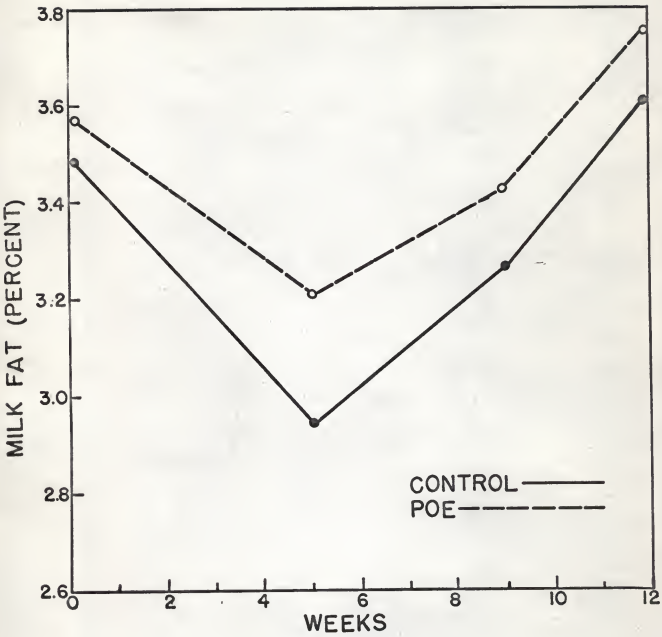

Figure 3. Effect of POE on average milk fat test per cow.

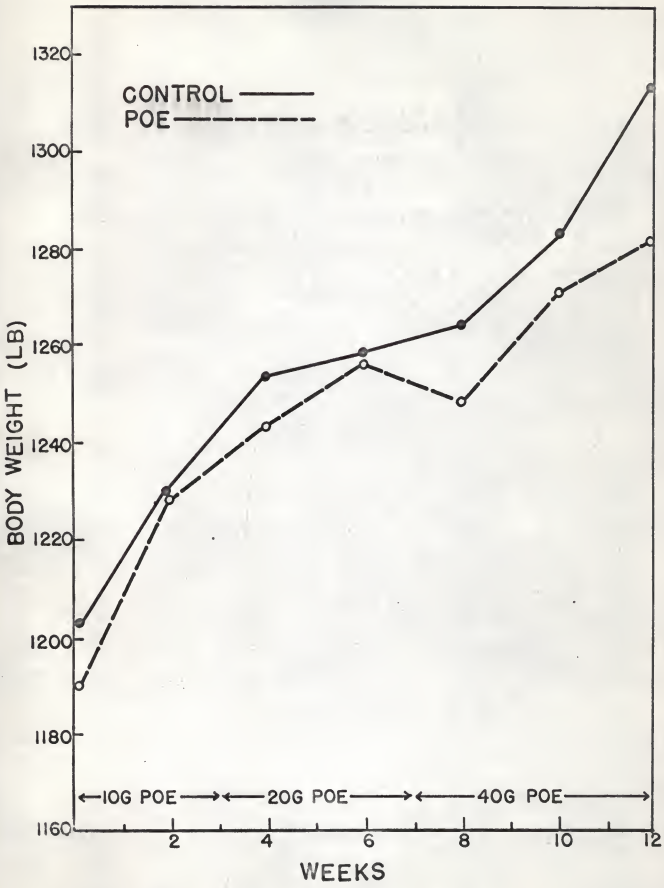

Figure 4. Effect of POE on average body weight per cow.

TABLE 1

Feed intake of lactating cows receiving either POE or the control ration (average per cow for 12 wk)

	POE	Control
	lb	lb
Grain fed	12,168	12,382
Grain refused	68	195
Grain consumed	12,100	12,187
Hay fed	9,156	8,988
Hay refused	1,423	891
Hay consumed	7,733	8,097

The cows receiving POE had a better conception rate (Table 2) than those in the control group. Since conclusion of the experiment, most of these cows have calved normally and there has been no indication of abortions or other abnormalities.

TABLE 2

Effect of POE on conception rate of lactating cows

	POE	Control
Number of times bred	9	12
Number of conceptions	6	6
Services per conception	1.5	2.0

The feeding of POE appeared to be without effect on general health and well-being of the animals. Two cases of mastitis occurred, one in a control cow and the other in a cow receiving POE.

Discussion

None of the results obtained in this experiment indicate any clear-cut evidence that POE has any deleterious effect on milk production, fat test, body weight, feed intake, reproduction, or animal health. In fact, POE appears to have a stimulatory effect on milk production. This observation is deserving of further study.

Cows receiving POE consumed approximately the same quantity of hay and grain as consumed by controls while producing more milk containing a higher percentage of fat than the control cows. Therefore, it is not surprising that the cows in the treatment group gained less weight than those in the control group. The increase in milk production observed during the first 2 weeks of the experiment for both groups was probably due to the high level of concentrates fed. Changing the cows' roughage from long hay and silage to chopped hay and decreasing the hay:grain ratio may have caused the sharp drop in milk fat test observed at the end of the experiment.

The data indicate that POE does not have a deleterious effect on reproductive efficiency or animal health.

Although palatability has been a problem in the use of many otherwise effective surfactants, the results of the feed intake study indicate that POE was readily acceptable by animals in this study. Until the cows became accustomed to eating the POE and control mixtures (usually within 7 days), they were mixed with the grain fed, but thereafter both were placed on top of the grain. Since the POE and control mixtures refused were included in the grain refusals, the feed intake data indicate that the POE mixture may have been more acceptable than the control mixture. Further work is needed to verify this assumption.

Since the level of POE was increased as the experiment progressed, any detrimental effect of POE should presumably be magnified because of the cumulative effect of the treatment. As the results are contrary to this presumption, the conclusion that POE does not deleteriously affect milk production, milk fat test, body weight, feed intake, or reproduction is clearly strengthened.

EXPERIMENT II

Experimental Procedure

Since the efficiency with which feed is utilized in the ruminant is largely dependent on rumen fermentation, it is essential that antifoaming agents used to prevent bloat do not adversely affect normal fermentation processes in the rumen. In this experiment, the effect of POE on fermentation was determined by measuring changes in rumen ammonia, rumen pH, rumen volatile fatty acids (VFA), lactic acid, and cellulose digestion.

Feeding and Management. Two sets of rumen fistulated identical twin cattle were fed alfalfa hay and salt ad libitum during a 2-week preliminary period. During the next 2 weeks, one member of each twin pair received in addition to the above ration, 1 lb per day of Supplement I (10 g POE per lb) and the other member received 1 lb per day of the control mixture (described on page 24). The treatments were reversed for each twin pair during the final 2 weeks of the trial. All animals were confined in stanchions throughout the experiment and were fed twice daily at approximately 12-hr intervals. Water for these animals was shut off 2 hr prior to and during sampling. The body weights of the animals in pounds were as follows: 04, 1056; 05, 1062; 18, 1043; and 19, 1034.

Collection of Samples. The fistulas of all animals were fitted with rubber sampling tubes which permitted withdrawal of a representative sample of rumen fluid without opening the fistula cap or disturbing the rumen contents. These sampling tubes were fitted on one end with a perforated metal tube to strain out large particles of rumen digesta. This metal tube was positioned in the ventral sac of the rumen about 12 inches below the fistula opening. The other end of the sampling tube extended outside the rumen through a hole in the fistula cap, and when not in use was kept closed with a screw-clamp. A stainless steel syringe was attached to the sampling tube to withdraw rumen fluid. Clean water was used to rinse the syringe and flush the sampling tube between samples. The first syringe full of rumen fluid was always discarded to prevent contamination or dilution of the sample.

Samples of rumen fluid for VFA, lactic acid, ammonia, and pH determinations were obtained 2, 4, and 6 hr after the morning feeding at weekly intervals throughout the experiment. On the next day rumen fluid used as inoculum for in vitro cellulose digestion was obtained 4 hr after the morning feeding. Rumen ammonia and rumen pH samples were ejected from the syringe into 4 oz wide mouth jars containing about 0.5 inches of mineral oil. Rumen fluid used for in vitro cellulose digestion was collected in half-pint milk bottles fitted with rubber stoppers. Samples for VFA and lactic acid determinations were placed in 6 oz polyethylene bags.

Determination of Rumen Ammonia. Determination of rumen ammonia was made as quickly as possible after the samples were collected. The modified method of Conway (1957) was used. To avoid contamination from the layer of mineral oil covering the rumen fluid, air was forced out through the tip of

the pipette before any rumen fluid was drawn into it. Tissue paper was used to remove any mineral oil which was adhering to the outside of the large-bore 1 ml pipette used to transfer the rumen fluid to the Conway plates. Determinations were made in duplicate for each sample. To standardize the end-point determination in the titration procedure, a blank was set up with each group of samples.

Determination of Rumen pH. After a small amount of rumen fluid was removed for the determination of rumen ammonia, the remainder was tested with a Leeds and Northrup potentiometer to determine pH. To avoid possible variables, all samples were stirred before pH readings were made and the meter's electrodes were rinsed with both ether and distilled water and wiped dry with tissue paper before testing the next sample.

Determination of Cellulose Digestion. The artificial rumen technique described by Baumgardt et al. (1962) was used to determine the effect of rumen fluid obtained from POE-fed and control animals on cellulose digestion *in vitro*. In each flask was placed 1 g finely ground alfalfa hay as substrate, 25 ml rumen fluid as inoculum, and 30 ml buffer. The flasks were incubated for 24 hr in a water bath shaker maintained at 39 C. At the end of the fermentation period, the contents of the flasks were dried in a forced air oven and cellulose determined by the method of Crampton and Maynard (1938).

Determination of Rumen Volatile Fatty Acids and Lactic Acid. The concentrations and molar proportions of VFA and lactic acid were determined using the procedure of Wiseman and Irvin (1957). Several modifications were made of the original method.

Solvents were made up using various percentages by volume of reagent grade acetone in Phillips 66 high purity, normal hexane rather than Skelly-

solve B. The percentages employed were as follows: 1%, 5%, 15%, 30%, and 50%. To prevent gradual removal of water from the column, concentrations of 15% and above were equilibrated as follows: 4 liters were stirred with 100 ml of 1:1 sugar solution, allowed to settle 2-3 hr, and filtered.

Columns were packed with sufficient absorbent slurry to give a depth of approximately 5 inches of compact material. Each column was used twice before the absorbent material was discarded. The cap material was made up of 8 parts celite plus 12 parts sodium sulfate (ammonium sulfate should not be used).

After the rumen fluid samples had been removed from the freezer and allowed to thaw, they were centrifuged in a size 1, model SBV International Centrifuge at 3,000 r.p.m. for 20 min and the supernatant was used for the VFA determinations. The cap material was used to absorb 1 ml of the centrifuged rumen fluid plus 4-5 drops of 50% sulfuric acid (V/V). After these ingredients had been thoroughly mixed in a small beaker, the mixture was placed on top of the column. Blanks and standards were prepared in a similar manner by substituting distilled water and standard acids respectively for rumen fluid.

The eluting solvents used were 1% acetone-hexane to elute valeric and butyric acids; 5% to elute propionic acid; 15% to elute acetic acid; and 30% to elute lactic acid. Since the concentration of formic acid in the rumen samples was too low to be separated, no 20% solvent was used.

To prepare the titrant, 25 g potassium hydroxide pellets were dissolved in 400 ml isopropanol by warming in a steam bath. The supernatant isopropanol-potassium hydroxide solution obtained by cooling and decanting this solution contained approximately 50 mg potassium hydroxide per ml. To make 1 liter

of approximately 0.01N titrant, 12 ml isopropanol-potassium hydroxide stock solution was mixed with 488 ml methanol and 500 ml isopropanol.

Seven columns were run simultaneously. The cap materiel placed on top of five of these columns contained rumen samples, one was a blank, and the other contained a standard acid solution to check the recoveries of each set of columns. If the recovery of standard acids deviated markedly from the values expected, the columns were discarded and the samples were rerun.

Results

Since graphical analysis of the rumen ammonia and rumen pH data indicated that averaging the values obtained during the two consecutive weeks of each period would not materially affect the results of the experiment, this manipulation was performed to condense the data so that the results may be more readily interpreted.

The rumen ammonia values (Table 3) during Period I show the expected close correlation between animals within a twin pair. During Period II, it appears that the feeding of 10 g POE may have caused rumen ammonia values to drop in animal 18. However, this did not occur with the other twin pair. In Period III, lower ammonia values appeared for the animals fed POE. However, when standard deviations were calculated, these differences did not appear to be real.

Rumen pH values (Table 3) were similar for all four animals in each period. Consequently rumen pH appeared to be unaffected by the feeding of POE.

During the first week of Period II, rumen ingesta from one twin (05) receiving POE resulted in greater cellulose digestion than its twin mate (04).

TABLE 3

Effect of POE feeding on rumen ammonia, rumen pH, and *in vitro* cellulose digestion

Animal no.	POE fed/ day	NH_3 per 100 ml rumen fluid[a]			Rumen pH[a]			Cellulose digested	
		2hr	4hr	6hr	2hr	4hr	6hr	1st week	2nd week
	(g)	(mg)	(mg)	(mg)				(%)	(%)
					Period 1				
04	0	18.9	17.6	12.0	6.6	6.6	6.7	43.7 ± 1.0	---[b]
05	0	25.9	16.3	14.4	6.6	6.7	6.7	45.9 ± 1.8	42.16 ± 0.7
18	0	27.3	15.3	10.0	6.6	6.4	6.8	43.1 ± 0.7	46.4 ± 1.2
19	0	28.4	14.8	10.6	6.7	6.7	6.7	43.8 ± 2.5	44.2 ± 1.8
					Period 2				
04	0	25.2	17.0	12.6	6.8	6.9	7.0	48.1 ± 0.5	44.3 ± 2.6
05	10	22.3	17.1	15.5	6.8	7.0	7.0	50.1 ± 1.6	44.9 ± 3.4
18	10	16.8	11.9	9.7	6.9	6.9	7.0	50.1 ± 0.6	46.4 ± 2.0
19	0	20.1	13.7	14.7	6.9	7.0	7.0	52.1 ± 1.1	46.8 ± 0.7
					Period 3				
04	10	17.3	13.6	12.0	6.8	6.9	7.0	43.9 ± 1.9	43.4 ± 0.8
05	0	18.1	15.3	12.3	6.7	6.8	6.7	45.5 ± 2.5	45.2 ± 2.3
18	0	15.8	13.2	8.3	7.0	6.8	6.9	45.3 ± 1.4	40.1 ± 1.2
19	10	15.5	10.8	8.3	7.0	7.1	7.2	44.9 ± 1.1	43.6 ± 0.8

[a] Each value is an average of the two values obtained at the end of the first and second weeks of each period.

[b] Animal off feed.

However, in the other twin pair (18 and 19) this effect was reversed. During the second week of Period II, cellulose digestion for both sets of twins was similar. During the first week of Period III, cellulose digestion was slightly lower for both animals receiving POE. However, during the second week of Period III, the digesta of one animal (19) receiving POE resulted in a greater digestion of cellulose than digesta from its twin mate (18). The digesta from the other animal (04) receiving POE resulted in a lower digestion of cellulose than its twin mate (05). Consequently neither a beneficial nor detrimental effect of POE on cellulose digestion is apparent.

A graphical analysis was made of the rumen VFA and lactic acid data (Table 4) and it was concluded that averaging the values obtained from 2, 4, and 6 hr samples would not affect the interpretation of results. Except for the unexplainable shift in the acetic acid and propionic acid ratios of animal 19 during the first week of Period I, the expected close correlation of VFA ratios between animals within the same twin pair is observed for the preliminary period. Ratios of rumen VFA during Period II are similar within twin pairs despite the fact that one member of each pair was receiving 10 g POE. During Period III, VFA ratios for one twin pair (04 and 05) remained similar. In the other twin pair (18 and 19), the animal receiving the POE showed a slight decrease in acetic acid with a corresponding increase in propionic during the first week of Period III, but the ratios reported for the second week were similar in both animals.

The total concentration of VFA (Table 4) was similar within twin pairs during the first week of the preliminary period, but varied during the second week. The low total concentration values found during the first week of

TABLE 4

Effect of POE feeding on rumen VFA and lactic acid

Animal no.	Gram POE fed/ day	Total concentration of VFA (mm/ml) 1st week	2nd week	Ratios of rumen VFA (%) 1st week Valeric	Butyric	Propionic	Acetic	Lactic	2nd week Valeric	Butyric	Propionic	Acetic	Lactic
							Period 1						
04	0	63.5	65.7	0.6	6.5	13.8	77.9	1.7	0.9	6.7	14.7	76.2	1.5
05	0	60.1	79.1	0.7	7.0	13.4	77.8	1.1	1.1	6.6	14.8	75.6	1.9
18	0	86.9	89.5	0.5	7.0	12.9	77.4	2.2	0.3	6.5	13.1	78.3	1.8
19	0	86.1	57.2	0.8	6.6	20.6	69.6	2.1	0.9	6.3	13.3	78.2	1.4
							Period 2						
04	0	18.0	41.4	1.0	7.3	16.5	74.8	0.4	1.7	5.0	12.3	79.1	1.9
05	10	26.6	57.3	0.7	6.1	15.1	76.2	1.7	1.8	5.3	13.3	77.1	2.0
18	10	23.3	57.9	1.2	5.9	14.0	74.1	4.7	0.7	5.4	17.2	75.0	1.7
19	0	18.4	64.9	1.2	6.2	14.2	74.5	4.0	1.3	5.2	16.2	75.2	2.2
							Period 3						
04	10	50.4	34.7	0.9	8.4	14.5	74.7	1.5	2.3	6.3	14.1	76.3	1.0
05	0	34.7	44.4	1.2	6.3	14.2	75.9	1.4	0.5	6.0	15.1	75.7	2.6
18	0	36.3	45.2	1.3	6.0	13.4	75.9	3.4	0.6	6.7	13.8	76.6	2.1
19	10	36.2	44.1	0.9	6.5	16.5	72.9	3.2	0.5	6.6	13.6	77.4	1.9

Period II suggest that the barn help may not have fed the animals the morning the samples were taken. Results for this week indicate the animals receiving POE have higher total concentrations of VFA, but during the following week, the value determined for one of the treatment animals (18) was less than its twin mate while the values for the other treatment animal (05) was greater than that of its twin. During Period III, similar total concentrations of VFA were observed for one twin pair (18 and 19). In the other twin pair (04 and 05), the animal receiving POE (04) had a higher total VFA concentration value than its twin during the first week of Period III, but had a lower value the second week. The total concentration and ratios of lactic acid were similar for all four animals in each period.

Discussion

Although the values reported for rumen ammonia, rumen pH, total concentration of rumen VFA and lactic acid, and cellulose digestion correlate very closely with the values that would be expected from rumen samples collected at a corresponding time from animals consuming an all hay ration, the rumen VFA ratios are slightly higher in acetic acid and slightly lower in both propionic and butyric acid than would normally be expected. Several VFA determinations were made using a gas chromatograph as a check against the column chromatography technique. The VFA ratios obtained by the two methods were nearly identical. The recovery rates of the standard acid samples indicate that all acids were being removed with the same degree of efficiency.

From the results obtained in the various phases of this experiment, it is difficult to find any clear-cut evidence that POE has any effect on rumen

fermentation. In each of the various trials, there are isolated cases where POE appears to have a slight effect, but there is also additional evidence which indicates that POE has the opposite effect or has no effect at all. There is, therefore, no substantial evidence in this experiment to indicate that POE has any effect on rumen ammonia, rumen pH, rumen VFA, rumen lactic acid, or in vitro cellulose digestion.

EXPERIMENT III

Experimental Procedure

Since residues from feed additives which appear in meat or milk cannot be tolerated, this experiment was designed to determine the fate of metabolic products of C^{14}-labeled POE.

Feeding and Management. A 7-year old Jersey cow weighing 810 lb and producing 15 lb of milk daily was obtained from the University dairy herd. The cow was confined in a stall in the climate controlled metabolism room in Call Hall at the University. The daily roughage ration consisted of 17 lb sorghum silage plus alfalfa hay fed ad libitum. The cow was milked at 6 AM and 6 PM each day and was fed 7 lb of herd concentrate ration prior to each milking. Carbon^{14}-labeled POE, diluted in 40 g unlabeled POE, was administered in a gelatin capsule by balling gun in the afternoon of the first day of the experiment.

Collection and Analysis of Samples. All urine and feces voided were collected daily and aliquots of each analyzed for C^{14} activity. Daily aliquots (10% of yield) of morning and evening milkings were combined and analyzed for C^{14} activity. On the first and last day only the milk from the evening and morning milkings, respectively, was analyzed.

The cow was sacrificed 9 days and 16 hr after the isotope was administered. Samples of blood, digesta, and tissues (Table 6) were analyzed for C^{14} activity.

The C^{14}-labeled POE[1] had a specific activity of 5.78 mc/g. With that specific activity, the detection of 100 dpm (about 30 count/min) above background would represent 0.75 of a nanogram of C^{14}-labeled POE. By analyzing 30 mg dry matter of milk, blood, tissues, digesta, or feces, it is possible to detect 25 parts POE per billion parts dry matter.

Samples of milk, blood, tissues, digesta, and feces were lyophilized and combusted using the Schöniger dry combustion technique of Kelly et al. (1961). The resulting CO_2 from 30-50 mg samples was absorbed in 1:1 ethanolamine-methyl cellosolve, and an aliquot of the absorbant was transferred to a counting vial containing the scintillator solution used by Jeffay and Alvarez (1961). Samples were counted in a Packard Tri-Carb Liquid Scintillation Spectrophotometer, Model 314ES.

All samples were lyophilized in duplicate, combusted in duplicate, and counted twice for at least 1 min when a counting rate greater than 10,000 count/min was obtained, or twice for 5 min for samples with a counting rate below 10,000 count/min. After sample counting rates were determined, C^{14}-benzoic acid (about 10,000 count/min) was added to each counting vial and the samples recounted for efficiency determination.

Urine was analyzed with no special preparation by using the dioxane scintillator solution described by Bruno and Christian (1961), which can incorporate 1 ml urine without forming two phases, even at 4 C. The same counting procedure used for lyophilized samples also was applied to urine samples.

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[1] C^{14}-labeled POE supplied by Smith Kline and French Laboratories.

Results

Data in Table 5 indicate that no milk sample had a count rate significantly above background. These results were obtained under conditions where a large quantity of C^{14}-labeled POE was administered to a cow whose daily milk yield was relatively low.

Data in Table 6 indicate that no C^{14} was deposited in the blood or tissues of the cow. Except for digesta in the omasum, only background count rates were detected for digesta in organs of the digestive tract.

Data in Table 7 show that of the C^{14}-labeled POE administered, 94.3% of the total C^{14} activity was excreted in the feces and 4.0% was excreted in the urine.

Discussion

Since no C^{14} was detected in milk, it may be concluded that POE is not transmitted to milk. Even if C^{14} had been detected in the milk, it would not necessarily indicate the presence of POE per se. Carbon^{14} could have appeared in the form of a metabolite of POE.

The digesta of the omasum had a count rate above background of 51 count/min with a large standard deviation. Even if this count was considered significant, it would not be unexpected because of the concentrating function of the omasum.

Previous studies conducted by Alexander et al. (1965) and Yadava et al. (1965) using C^{14}-labeled alfalfa showed that C^{14} first appeared in the feces 12 hr after feeding. Therefore, the excretion pattern observed in Table 7 appears normal. It is apparent that POE is poorly absorbed from the digestive

TABLE 5

Carbon^{14} activity of milk from Jersey cow administered C^{14}-labeled POE

Days after administration of C^{14}-labeled POE	Count rate of milk
	count/min
1	33 ± 1
2	37 ± 2
3	37 ± 1
4	33 ± 1
5	33 ± 2
6	31 ± 1
7	32 ± 3
8	31 ± 2
9	30 ± 1
10	31 ± 1
Background	36 ± 2

TABLE 6

Carbon^{14} activity of blood, tissues, and digesta of Jersey cow nine days and 16 hr after administration of C^{14}-labeled POE

Sample analyzed	Count rate
	count/min
Digesta	
Rumen	26 ± 2
Reticulum	32 ± 2
Omasum	87 ± 50
Abomasum	36 ± 3
Small intestine	33 ± 2
Blood	32 ± 2
Tissues	
Thyroid	30 ± 1
Pituitary	21 ± 4
Liver	43 ± 3
Kidney	35 ± 2
Pancreas	40 ± 5
Spleen	32 ± 2
Rumen fat	32 ± 4
Peritoneal fat	30 ± 1
Lung	31 ± 3
Brain	30 ± 4
Rumen mucosa	30 ± 1
Longissimus dorsi	32 ± 3
Heart	33 ± 1
Mammary tissue	34 ± 5
Bone chip from humerus	25 ± 2
Bone marrow from humerus	30 ± 4
Background	36 ± 2

TABLE 7

Carbon^{14} activity of urine and feces from Jersey cow administered C^{14}-labeled POE

Days after administration of 8029 μc POE	Feces	Urine
	μc	
1	335.3	174.1
2	4192.0	112.9
3	1875.0	24.9
4	907.5	5.6
5	170.1	1.3
6	89.2	1.6
7	0.6	0.4
8	0.3	0.4
9	0.1	0.2
Total	7570.1	321.4
Recovery (%)	94.3	4.0

tract and that the small quantity absorbed is rapidly eliminated in the urine. From the extent of elimination of POE in feces and urine, it is not surprising that no C^{14} activity was present in milk or body tissues.

It may be concluded that POE is not eliminated in milk secreted from a few hours to 10 days following the administration of POE. Also, no residues of POE are present in body tissues 10 days following its administration.

CONCLUSIONS

1. Feeding POE to lactating cows does not deleteriously affect milk production, fat test, body weight, feed intake, reproduction, or animal health. In fact, POE appears to have a stimulatory effect on milk production.

2. Feeding POE to two sets of rumen fistulated identical twin cows had no apparent effect on rumen pH, rumen ammonia, rumen VFA and lactic acid, or *in vitro* cellulose digestion. Therefore, POE appears to have no detrimental effect on fermentation in the rumen.

3. Most of the C^{14}-labeled POE administered was eliminated in the feces and urine. None was excreted in the milk or deposited in the tissues. This suggests that the products from animals fed POE are safe for human use.

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APPENDIX

APPENDIX TABLE 1

Effect of POE on milk and fat production, feed intake, and reproduction

Cow no.	Milk production	Fat production	Grain consumed	Hay consumed	Services/ conception
	(lb)	(lb)	(lb)	(lb)	
			POE		
A129	3719.8	126.6	2168.2	1561.8	1
A132	3929.1	126.9	2350.9	1515.6	1
A135	2901.2	119.6	1842.0	1165.2	1
1590	3292.8	125.8	1943.6	1115.0	3
1330	3581.7	101.2	1938.1	1071.4	2
270C	4127.7	138.7	1857.1	1304.5	1
			Control		
A138	3850.0	132.4	2333.3	1553.9	1
A162	4548.8	154.7	2587.7	1538.1	2
A140	2914.2	83.3	1682.5	842.3	2
A144	2821.1	94.2	1927.5	1482.8	2
130D	3558.4	113.5	1810.6	1370.9	2
261C	2666.2	92.4	1845.0	1309.2	3

RESIDUE STUDIES WITH A NONIONIC SURFACTANT USED FOR BLOAT CONTROL IN DAIRY CATTLE AND ITS EFFECT ON RUMEN FERMENTATION, FEED INTAKE, AND MILK PRODUCTION

by

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An acceptable bloat preventive agent must not only control bloat effectively, but must have no adverse effects on the animal's ability to grow, produce, reproduce, and maintain itself. Also, it must not be eliminated in milk or be found in body tissues.

The object of this research was to test the effect of a nonionic surface active agent on milk production, milk fat content, feed intake, rumen fermentation, body weight, and reproduction. The surface active agent is polyoxypropylene polyoxyethylene block polymer (POE) used in the control of legume bloat.

Lactating cows fed varying levels of POE (10-40 g per day) produced slightly more milk with a higher average milk fat content than control cows. The control cows consumed a little more grain and hay than the cows receiving POE, but refused more of the grain fed. Palatability of POE was not a problem. Slightly larger weight gains were made by the control cows. Conception rates were somewhat higher in the cows receiving POE. Despite the slight differences observed, none of the changes was of sufficient magnitude to justify their being attributed to the feeding of POE.

To determine the effect of POE on rumen fermentation, rumen fluid was collected from two sets of fistulated identical twin animals, analyzed for ammonia, pH, lactic and volatile fatty acids, and tested in vitro for cellulose digestion. The bloat preventive agent POE had no apparent effect on either the rumen fermentation products tested or in vitro cellulose digestion.

A residue study using C^{14}-labeled POE was conducted to determine whether POE is eliminated in milk or remains in tissues when fed to a lactating cow. No C^{14} appeared in the milk for 9 days following administration of the labeled drug. Most of the C^{14} was eliminated in the feces. No C^{14} was present in blood, organs, or tissue examined after slaughter.

The data obtained in these studies indicate that POE can be used for bloat control in dairy cattle without any problem of residues appearing in milk or body tissues and without adversely affecting rumen fermentation, feed intake, production of milk, health, or reproduction.